strains gave beta reactions and "double zone" phenomenon on 5.0 per cent horse blood agar. The post-operative meningitis did not respond to sulfanilimide therapy despite maintenance of satisfactory blood levels. The group D strains were classified by precipitation and agglutination of both the protein-like group and the carbohydrate type-specific components. This method will be described in detail elsewhere. Alpha, beta and gamma strains are frequently encountered in this group. The strain isolated from the fatal peritonitis was heat-resistant. Biological characteristics could not be correlated with serological type. The strains isolated from sub-acute bacterial endocarditis gave alpha reactions on 5.0 per cent horse blood agar. In one case, the micro-organism was recovered from the blood cultures over a period of four weeks. Three of the cases proved fatal.

These non-group-A strains represent but a small percentage of the total number of hemolytic streptococci isolated from human infection during these studies. They are of interest in that many of them were associated with fatal infections.

The diversity of clinical diagnoses encountered in this small series is in accord with the observation that non-group-A streptococci are frequently associated with non-respiratory streptococcal infections (Rantz and Kirby (1942)).

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DIGESTION OF CASEIN BY STAPHYLOCOCCI ON MILK AGAR CONTAINING SERUM

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Proteolytic zones surrounding the colonies of staphylococci are occasionally seen on the milk agar medium used in this laboratory to determine chromogenesis. These zones are for the most part narrow and indistinct and require more than 24 hours to appear. A marked increase in casein digestion was observed when the medium was enriched with serum. Tests were run with 175 strains of staphylococci of human origin to compare caseinolysis on serum milk agar with the coagulase and fibrinolytic activity of these organisms. The cultures were isolated from various pathologic specimens and from the nose, throat, scurf and urine.

Serum milk agar was prepared by adding 30 ml. of skim milk to 20 ml. of distilled water containing 1.5 g. of agar. The milk and the agar solution were sterilized separately at 121°C. for 10 minutes and were mixed with 50 ml. of serum

after cooling to 50°C. Pooled human serum obtained from serology specimens and sterilized by Seitz filtration was employed. Inoculations were made on the surface of the medium which was dispensed in petri dishes and the cultures were incubated at 37°.

Coagulase production was determined with citrated rabbit plasma which was added in 0.5 ml. amounts to 0.2 ml. of tryptose broth containing a suspension of the organisms to be tested. Overnight growths of cultures grown on a solid medium were used for this purpose and suspensions were made to approximate the opacity of an 18-hour broth culture of staphylococci. The tests were incubated at 37° and examined for coagulation at hourly intervals for 3 or 4 hours and again after overnight incubation.

Tests for fibrinolysis were run by the heat-precipitated fibrinogen method of Christie and Wilson 1941. Citrated rabbit plasma and the medium proposed by Chapman 1942 were used. The medium was allowed to harden in petri dishes and was inoculated in the same manner employed for serum milk agar.

Zones of clearing surrounding the growth of caseinolytic strains appeared on the serum milk medium usually after 24 hours of incubation but occasional

TABLE 1

Comparison of coagulase, fibrinolysis and caseinolysis tests with 175 strains of staphylococci

COAGULASE	FIBRINOLYSIS		CASEINOLYSIS	
	Positive	Negative	Positive	Negative
Positive (102)	91	11	82	20
Negative (73)	1	72	2	71
	Positive (92)		81	11
	Negative (83)		3	80

cultures required 48 hours to become positive. The zones of fibrinolysis on heated plasma medium were for the most part larger and were almost always present after overnight incubation. Plaque-like areas of clearing which protruded from the periphery of the zone of fibrinolysis and which were sometimes entirely separate from this zone were frequently seen.

The results of comparative tests presented in table 1 show that the ability of staphylococci to produce fibrinolysis and caseinolysis is similar to their coagulase activity. Since the coagulase reaction is generally believed to be the most reliable test for pathogenicity among staphylococci these results are of interest. Whether or not the disagreement in reactions shown by some culture strains may be significant in determining some factor of pathogenicity awaits further study. The mechanism of casein digestion which occurs in a medium rich in serum was not determined.

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